Long term effects of double charged hemihypoglossal-Facial Nerve Neurorrhaphy on the histological structure of orbicularis oculi muscle

Khaled I. Barakat¹, Mohamed M. Rabea¹, Dalia A. El Waseef², Hamed Gad³

Department of Oral and Maxillofacial Surgery Faculty of Dentistry Minia University¹, Department of Histology and Cell Biology Faculty of Medicine Ain Shams University²

INTRODUCTION

Facial paralysis implies a significant affection to the quality of life. It is a complex neuromotor and psychomotor process linking physical expression with emotion. It leads to negative aesthetic, physiological and psychological effects¹,².

The etiology of facial palsy (FP) is idiopathic, traumatic or neoplastic, or may be caused by their treatment sequelae. Therefore, treatment is based on the surgeon’s experience and patient’s profile, including; age, general condition, prognosis, timing of reanimation and gaps length. The gaps length and accessibility of proximal stump is the main factor that determines the technique of repair modality¹.

Modalities of facial nerve repair are classified according to gap length into direct anastomosis (end to end) [ETE] which is used in small gaps or indirect anastomosis by using nerve grafting in large gaps. Grafts are further classified into free cable nerve graft as sural nerve graft or nerve transfer graft as masseteric and hypoglossal nerve grafts²,³.

However, grafting requires a long neurotization time which patients AND METHODS permanently alter the motor end plate function causing muscle fibrosis².

In order to preserve the muscle function, several methods of indirect motor reinnervation have been proposed. One of the most popular is the hypoglossal–facial neurorrhaphy⁴.

Although it proved its effectiveness in terms of reducing the period of interruption of the conduction of signals to muscles and preserving the nerve receptors but unfortunately it suffered a high morbidity due to sacrificing the hypoglossal nerve function. Nevertheless, the classic hypoglossal–facial neurorrhaphy is associated with unavoidable hemihypoglossal atrophy and persistent mastication, speech and/or swallowing problems that interfere with daily life⁵,⁶.

Accordingly, many variants of this procedure have been developed over the past 2 decades to reduce morbidities of the tongue function while preserving its short onset

ABSTRACT

Objective: to assess the effect of Hemi Hypoglossal-Facial nerve neurorrhaphy (HFN) with double charge "supercharge" technique on the structure of orbicularis oculi muscle (OOM) fibers in goats.

Material and method: The current research was carried on six goats where the right side acted as experimental while the left acted as control without any intervention. The right Facial nerve (FN) was intentionally transected and immediately repaired using an ipsilateral end to end hemi hypoglossal nerve (HHN) neurorrhaphy to the distal facial trunk and end to side to its proximal end "supercharge". One year later animals were anaesthetized and samples were bilaterally harvested from (OOM). Both groups were histologically and clinically assessed and compared.

Results: Double charged neurorrhaphy technique revealed the preservation of the normal histological pattern of muscle fibers, arrangement, shape and diameter. However, collagen fibers were significantly increased around the muscle fibers (P < 0.05). On the other hand, the nuclei retained their normal number and sites.

Conclusion: HHFN with supercharge histologically preserved the structure of the OOM fibers reflecting the excellent clinical outcome of the technique.

Key Words: facial nerve neurorrhaphy, hemihypoglossal nerve neurorrhaphy, super charge technique, goats model, histology of orbicularis oculi muscle.

Received: 2 March 2020, Accepted: 28 April 2020.

Corresponding Author: Khaled I. Barakat, Professor at Oral & Maxillofacial Surgery, Department. Faculty of Dentistry. Minia University, Egypt, Tel: +20222677077, Mobile: 01222324596, E-mail: send2kbarakat@yahoo.com.

ISSN: 2090-097X, July 2021, Vol. 12, No. 2
of reinnervation and allowing a faster rate of muscle movement\textsuperscript{3,7}.

In order to overcome this predicament, surgeons induced the hemi or partial hypoglossal fascicle repair, which ultimately decreased the technique morbidity while maintaining the muscle trophism. Moreover, facial and hypoglossal nerves have many close similarities; their motor cortex shows a cortical topographical proximity, they also contain myelinated motor fibers with comparable fascicular anatomy and they both receive afferent input from the trigeminal reflex arcs. Furthermore, they both act synergistically in the coordination of some mimetic movements. All these comparable factors provide excellent basis for providing the same axonal charge\textsuperscript{4,6}.

In 1879 Drobnik was the first surgeon that performed nerve transferring to reinnervate facial muscle with neurorrhaphy between hypoglossal and facial nerves. In 1901 Körte and Bernhardt carried out their first hypoglossal facial anastomosis by interpositional nerve graft. In 1932 Balance and Duel emphasized direct ETE neurotization by complete transection of HN\textsuperscript{9,10}.

In 1979 Conley and Baker, reviewing the HHFN on 137 cases and recording good results\textsuperscript{11}. In 1991, May et al refined and actually named interpositional –jump graft technique in the half of the HFN\textsuperscript{12}. In last two decades, some modifications of the HFN have also been proposed in the literature\textsuperscript{13}. In 1873 Letievant was described alternative technique of FN rehabilitation surgery based on the double innervations of the paralyzed mimetic muscle. In 2004 Isaacs and colleagues have explained a supercharge neurorrhaphy on the experimental\textsuperscript{14}. In 2007, Fujiwara T, Matsuda K, et al proposed the concept of supercharge by aiming to conduct more axons in the nerve repair thus achieving faster and stronger conduction\textsuperscript{15,16}.

The technique involves the use of a hemihypoglossal fascicle to neurorhaphy the distal facial stump as an end-to-end neurorrhaphy while in the same time repairing both the facial proximal and distal stumps via an interposing nerve graft. Thus, adding more conduction from the proximal facial via the graft to its distal end\textsuperscript{14}.

Unfortunately, this method also suffers from the shortcoming of location a neighboring motor nerve that can be connected to the free nerve graft.

In an attempt to improve the technique, spare the need of a nerve graft and allow more immediate axon conduction we modified the super charge technique as follows:

We used the donor hypoglossal fascicle to conduct the distal facial stump as an end-to-end neurorrhaphy while anastomosing the proximal facial stump to the hypoglossal fascicle as an end to side repair (Figure.1).

We thus made use of the conduction from both the proximal facial and hypoglossal fascicle to augment the axon conduction of the fascicle to the distal facial stump.

Several methods have been used to evaluate the efficacy of the various nerve neurotisation techniques including physiological test EMG, clinical test, magnetic resonance imaging and histological assessment. However, all these assessment methods depend on measuring and evaluation of the conduction strength either directly or by stimulating the affected muscle\textsuperscript{17}.

Clinical evaluation is important for the patient outcome and satisfaction. However, all the above mentioned methods cannot accurately determine the number or percentage of viable and intact muscle fibers. Histological assessment is unique in determining the structural changes that occurred in the muscle fibers due to loss of innervations.

**MATERIAL AND METHODS**

This study was conducted on six local goats. All parameters were kept constant; race, age, weight and ways of nutrition for unification of factors to avoid any change health and healing or regeneration. Goats were approximately 24 months old, females and their weight 15 to 20 kg. All the goats were selected from Minia university animal house, Faculty of agriculture according to Faculty animal care protocol. General and physical examination was done.

Housing of 4X4 meters repaired daily nutrition regimen containing of grain rice straw and water were provided. The study followed the guideline for ethical conduct in the care and use of non-human animals in research and also the World Medical Association declaration of Helsinki.

**Animal grouping**

The study composed of two separate phases.
Phase one
Preoperative preparation

The goats fasted for 12 hours preoperatively and the surgery was done under standard prepping and draping technique. The left side acted as control side without any surgical intervention, while the right side acted as experimental.

Anesthesia technique

The procedures were carried out under Full Intravenous Anesthesia (TIV A) according to the Center for Veterinary Health Science Guidelines. Preanesthetic sedation by the sedative IV e.g. (xylazine 0.3 mg / kg) 20 minutes before anesthesia then: Intraperitoneal injection to induce anesthesia accomplished by combinations of Ketamine-Xylazine (ketamine 4 mg / kg 0.2 mg / kg Xylazine). TIVA triple drip solution combines one-liter normal saline with xylazine 100 mg and ketamine 200 mg at a rate of 0.5 to 2 ml / kg / hr.

Surgical procedure

Dissection of FN trunk and its zygomatic (ZFN) branch was first performed followed by dissection of HN from beneath the posterior digastric muscle up to its most proximal part before pricing towards the tongue. The distance between the HN at the angle of the mandible and ZFN was measured to determine the exact HN fascicle length needed to reach the ZN. A HN fascicle was cut according to the pre-determined length from anterior to posterior compromising around 40% of the HN diameter and remained pedicled posteriorly to the HN trunk at the angle area. Attention was then turned towards the ZFN which was transected just close to its origin from the FN trunk. The fascicle was then superiorly transposed and sutured as an ETE to the remaining distal part of ZFN. Meanwhile, the proximal part of ZFN at its origin was sutured as an ETS to the transposed fascicle. (Figure 2)

Clinical evaluation

Animals were evaluated clinically at 1, 3, 6 and 12 months based on a scale of closure of the eye and a blinking observation of the reflection closure (Table 1).

To standardize the stimulation we used two stimulation tools, firstly air inflation (10 ml syringe) (fig 3) on about 1cm away from the eyes to trigger the blinking reflex, and a cotton bud (Figure 4) to observe the actual closure. Tongue was tested for deviation, pricking reflex and atrophy or abnormalities.

<table>
<thead>
<tr>
<th>Score</th>
<th>Movement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No movement</td>
</tr>
<tr>
<td>2</td>
<td>Contraction /no closer</td>
</tr>
<tr>
<td>3</td>
<td>50% closer</td>
</tr>
<tr>
<td>4</td>
<td>75% closer</td>
</tr>
<tr>
<td>5</td>
<td>Complete closer</td>
</tr>
</tbody>
</table>

Table 1: scale of closure of the eye and a blinking observation of the reflection closure

Phase two

Harvesting of the OOM was executed under the same standard anesthetic procedure. The upper eyelids were bilaterally shaved and similarly marked for harvesting the OOM. A 3 cm incision was accomplished 1 cm above the lid margin, dissection then continued subcutaneously over the muscle just above the lashes to separate the muscle from the skin. The superior border of the OOM was cut and dissected from its underlying structures down to the lid margin and cut as a specimen with sharp scissors parallel to the muscle fibers (Figure 5).

Specimen was fixed under moderate tension in 10% buffered formalin in marked specimen container. Fixation was followed by dehydration, clearing and embedding in paraffin. Serial sections of 5 μm thickness were cut and stained with Haematoxylin and Eosin (H&E) as well as Mallory’s trichrome stain to examine the OOM fibers from a longitudinal section (LS) and transverse section (TS).

Morphometric and statistical study:

An image analyzer computer system Leica Qwin 500, UK connected to a Leica DM2500 microscope (Wetzlar, Germany), at the Department of Histology and Cell Biology, Faculty of Medicine, Ain Shams University, was used to measure the area % of collagen fibers (~40 power lens) in Mallory’s trichrome stained sections. Measurements were done in five non overlapping fields of two serial sections from all animals in each group. The mean of the previous parameter ± SD and the Student-T test were measured by SPSS, 21 programs (IBM Inc., Chicago, IL, USA). The significance of data was determined by $P$ value as $P < 0.05$ was considered significant.
RESULTS

The clinical results in (phase one) showed a primary decrease in the mean eye blinking reflex at one month postoperative then gradual increase to reach a score close to normal range at 6 month which remained constant at phase 2 one year postoperative. The tongue didn't show any weakness, abnormalities in function or reflexes between both sides.

The results of Mallory’s trichrome stained in (LS) and (TS) of the control group showed few or thin collagen fibers separating muscle fibers (Figure. 6). Whereas; in the study group there was apparent increase in amount of thick collagen fibers surrounding the muscle fibers (Figure.7). The H&E stained (LS) of the OOM in control group showed a parallel arranged muscle fibers which appeared cylindrical in shape, with nearly the same diameter. The muscle fibers had acidophilic sarcoplasm and well-defined transverse striations. Muscle fibers were seen separated by thin layer of connective tissue. Each muscle fiber had multiple elongated nuclei, seen peripherally under the sarcolemma (Figure. 8). On the other hand, the study group showed almost preserved parallelism of muscle fibers that appeared cylindrical in shape, with nearly the same diameter. Most of the muscle fibers were acidophilic. Some disorganized muscle fibers were seen among other apparently normal fibers. The disorganized muscle fibers appeared pale stained and without apparent transverse striations. Some areas showed aggregation of mononuclear inflammatory cells and congested blood vessels. Other areas showed normal, well organized muscle fibers (Figure 9).

The H&E stained (TS) sections of the control group showed bundles of muscle fibers separated by connective tissue. Muscle fibers were seen separated by thin layer of connective tissue. Each bundle was formed of group of polygonal, acidophilic muscle fibers with peripheral nuclei (Figure 10).

Conversely, the study group showed muscle fibers with irregular shapes and size. Mononuclear inflammatory cells were also seen within the connective tissue surrounding the muscle fibers (Figure 11). (Table 3 and 4)

| Table 2: Blinking reflex of OOM by time |
|-------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                  | N   | Mean | Std. Dev | Std. Error | Min | MAX |
| Pre-op           | 6   | 4.5000 | 0.53452 | 0.18898 | 4   | 5   |
| 1 M/Post-op      | 6   | 1.25  | 0.4629  | 0.10298 | 4   | 5   |
| 3M/Post-op       | 6   | 2.25  | 0.7071  | 0.12500 | 4   | 5   |
| 6M/Post-op       | 6   | 4.375 | 0.5175  | 0.16366 | 4   | 5   |
| 12M/Post-op      | 6   | 4.375 | 0.5175  | 0.16366 | 4   | 5   |

<p>| Table 3: Percentage of collagen area between groups |
|-------------------|-----------------|-----------------|-----------------|
|                   | Mean area % of collagen | SD | SEM |
| Control           | 3.24 ± 0.8      | 0.3            |
| Study             | 23.9 ± 5.5*     | 1.5*           |</p>
<table>
<thead>
<tr>
<th>Light microscopic examination on OOM</th>
<th>Haematoxylin and Eosin (H&amp;E)</th>
<th>Mallory’s trichrome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td><strong>LS</strong></td>
<td><strong>TS</strong></td>
</tr>
<tr>
<td><strong>study</strong></td>
<td><strong>control</strong></td>
<td><strong>study</strong></td>
</tr>
<tr>
<td><strong>parallel</strong></td>
<td><strong>Some disorganized fibers</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Arrangement</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>shape</strong></td>
<td><strong>cylindrical</strong></td>
<td><strong>irregular</strong></td>
</tr>
<tr>
<td><strong>Staining</strong></td>
<td><strong>acidophilic</strong></td>
<td><strong>red</strong></td>
</tr>
<tr>
<td><strong>branching</strong></td>
<td><strong>some</strong></td>
<td><strong>red</strong></td>
</tr>
<tr>
<td><strong>diameter</strong></td>
<td><strong>Uniform</strong></td>
<td><strong>Apparentlly decreased</strong></td>
</tr>
<tr>
<td><strong>transverse striations</strong></td>
<td><strong>Well defined</strong></td>
<td><strong>in apparent</strong></td>
</tr>
<tr>
<td><strong>destruction</strong></td>
<td><strong>no</strong></td>
<td><strong>Few &amp; pale</strong></td>
</tr>
<tr>
<td><strong>shape</strong></td>
<td><strong>elongated</strong></td>
<td><strong>flattened</strong></td>
</tr>
<tr>
<td><strong>size</strong></td>
<td><strong>normal</strong></td>
<td><strong>normal</strong></td>
</tr>
<tr>
<td><strong>site</strong></td>
<td><strong>Peripheral</strong></td>
<td><strong>Peripheral</strong></td>
</tr>
<tr>
<td><strong>number</strong></td>
<td><strong>multiple</strong></td>
<td><strong>multiple</strong></td>
</tr>
<tr>
<td><strong>Collagen fibers (Or C.T.)</strong></td>
<td><strong>Thin C.T.</strong></td>
<td><strong>Blue</strong></td>
</tr>
<tr>
<td><strong>Inflammatory cells</strong></td>
<td><strong>no</strong></td>
<td><strong>few</strong></td>
</tr>
<tr>
<td><strong>Congested blood vessels</strong></td>
<td><strong>no</strong></td>
<td><strong>Few</strong></td>
</tr>
</tbody>
</table>

Table 4: Histological comparison between normal and supercharged OOM
Figure 1: Schematic rendering of surgical techniques for supercharge concept. A: proximal part of zygomatic branch of F.N, B: distal part of zygomatic branch of F.N, C: H.N, D: hypoglossal dissected fascicle, E: End of HN fascicle-to-end of distal FN neurorrhaphy [ETE], F: End of proximal FN-to-side HN fascicle neurorrhaphy [ETS].

Figure 2: Operative view of the supercharge technique A: End-to-end neurorrhaphy of hypoglossal fascicle with the distal end of FN/ B: end-to-side with the proximal trunk of FN. C: hemi- hypoglossal fascicle D: marginal mandibular N.

Figure 3: Air inflation by 10 ml syringe.
Figure 4: Cotton bud for test blinking of eye.

Figure 5: Biopsy taken specimen

Figure 6: Photomicrograph of the (TS) in OOM in the (control group) showing CT around bundles of muscle fibers. Notice the few, thin collagen fibers (wavy arrow) between individual muscle fibers (M). Mallory’s trichrome × 400

Figure 7: Photomicrograph of the (TS) in OOM in the (study group) showing thick CT around bundles of skeletal muscle fibers and between individual muscle fibers (M). Mallory’s trichrome × 40

Figure 8: Photomicrograph of the (LS) in OOM in the (control group) showing parallelly arranged muscle fibers (M). The fibers appear acidophilic, cylindrical in shape, some fibers are branching (*). Muscle fibers are separated by thin layer of connective tissue (CT). Notice the prominent transverse striations (▲). Each muscle fiber has multiple elongated nuclei (↑), seen peripherally under the sarcolemma. H&E × 400

Figure 9: Photomicrograph of the (LS) in OOM in the (study group). Most of the muscle fibers (M) are acidophilic, cylindrical in shape, with multiple peripheral elongated nuclei (↑). Notice the presence of some destructed, pale stained muscle fibers (*). H&E × 400
DISCUSSION

Facial paralysis due to trauma, tumor resections or other causes had several devastating effects, especially on the OOM that leads to inability of closing the eyelids. Consequences of lagophthalmos include; eye redness, corneal ulceration, conjunctivitis, insect bites of the eye tissues and dryness which subsequently cause loss of vision.

In order to save the OOM function it is imperative to prevent its fibrosis by allowing immediate return of axonal charges. Re-innervation of facial nerve injury using free nerve grafts requires extended time to achieve axonal conduction which in turn result in permanent fibrosis of the muscle fibers. Nerve transfer techniques were developed to accelerate the nerve conduction, preserve motor end plates and prevent muscle fibrosis. However, those techniques suffered donor site morbidity and / or inadequate number of axons to conduct appropriate impulses.

In the current study we used a new modification of the supercharge neuroraphy technique of the facial nerve via a hemi hypoglossal motor nerve transfer. The technique relied on two main concepts; the first is to accelerate the nerve conduction by replacing the usual free nerve grafts by a viable HHN fascicle to ensure the immediate return of axonal charges. The second is to induce double nerve conduction (supercharge) from both the HHN fascicle and the proximal distal ZFN stump via the HHN fascicle in order to provide the OOM with huge axonal impulses than its normal counterpart. This supercharged nerve is supposed to substitute the loss of impulses due to nerve injury. We selected the hypoglossal nerve as an external source of facial nerve recovery because of its adjacent anatomical location, neuronal brain plasticity and ideal caliber. The large caliber offers a tremendous amount of nerve commands due to the large number of axons which contributes to preserving the muscle function.
Moreover, the use of HHN precluded the tongue morbidities that occurred with the total translocation of the HN. In the current study the tongue function was not affected in all goats of both groups. We selected local goats as an experimental animal in the current study because the larger size of goats when compared to small rodents allowed the usage of magnifying loupes to perform the supercharge neurorrhaphy instead of the complex surgical microscopes. Moreover, the close anatomical and histological similarity between the human and goat OOM together with their corresponding size offered a reliable comparison (Figure 12 and 13)\(^\text{[20]}\).

Regarding the assessment methods we used two different techniques in phase one we relied on the clinical assessment that measured the frequency of vertical eye movement opening and closing by time. It was important to obtain a simple, precise, and reliable evaluation method to assess the clinical condition of the eye blinking in goats. In reviewing the research, we based our assessment on the eyelid movement control scale table\(^\text{[29]}\). Clinical results showed primary decrease in the eye blinking reflex at one month postoperative due to nerve injury.

Then it gradually increased at 3 month and reached a constant score at 6 month and one year recording a score that is a very close to the control group thus reflecting the efficacy of the modified supercharge technique. Despite the good clinical results that were achieved by the eye blinking reflex score, this method is not considered a precise score because the excessive movement of the goats during the assessment affected its reliability. Thus, in phase 2, by the end of the study we added the histological assessment as a unique and highly reliable method in assessing the impact of the injury and the ability of the modified supercharge technique to re-innervate the muscle quickly and preserve its structures. Other studies also documented the histological assessments as an accurate method to assess the structures of fibrosed muscles after one year of denervation \(^\text{[17, 22]}\).

Up to our knowledge our study was unique in assessing the histological structures of the OOM that was reinnervated using a supercharge technique. 24 Fujiwara et al used the histological evaluation to assess the supercharge technique on the (gastrocnemius muscle) and proved that the histological assessment is a reliable technique in evaluating the supercharge technique\(^\text{[23, 24]}\).

The current histological results proved the efficacy of the supercharge technique. Supercharge technique increases the number of regenerating axons, which assume that the same muscle is fired by two different nerve signals\(^\text{[29]}\). Because of earlier reinnervation of the target muscle, supercharging not only increases regeneration but also possibly protects the muscle. A gradient in muscle fiber size appeared to be present, whereby the fiber size increased as a function of the distance from the eyelid margin \(^\text{[27]}\).

Long-term muscle denervation is characterized by three phases: an early period of rapid increase in muscular atrophy; an intermediate phase in which the atrophic mechanism is slowed down; and a final phase in which the gross muscle mass tends to be stable; a diminishing number of myofibers, replaced by fibrous and adipose tissues. The normal fascicular muscle architecture is lost at 9-11 months of permanent denervation, and interstitial tissue, which includes adipocytes and collagen sheets, increases dramatically at the expense of the myofibers\(^\text{[28]}\).

However, the current study showed that the architecture of the OOM was almost preserved after one year of complete cutting of the facial nerve followed by immediate supercharge neurorrhaphy. Moreover, the minimal changes that occurred in the histologic picture are considered within the early phase of denervated muscle degeneration according to Nicoletta et al. classification\(^\text{[30]}\). The technique is not devoid of shortcomings, it is only suitable for conditions where the proximal FN is present and can be easily sutured to the HN fascicle. The study could have benefited from inclusion of a negative control group in which the FN or the ZFN is transected and left for spontaneous total fibrosis.

**CONCLUSION**

HHFN supercharge technique is an efficient method to re-innervate the OOM as shown by its excellent functional clinical results and its histological ability to preserve normal architecture and structure of the muscle despite, the presence of mild insignificant variations.

**ACKNOWLEDGEMENTS**

The authors thank Dr. Mohammed Elkhouly for sharing his thesis contribution in phase one of the study.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**REFERENCES**


29. DeFaria SD, Testa JR, Standardization of techniques Used in facial nerve section and facial movement evaluation in rats. Brazilian Journal of Otorhinolaryngology,